



UNIVERSITI PUTRA MALAYSIA

**CRYOPRESERVATION OF EXCISED EMBRYOS OF RAMBUTAN
(NEPHELIUM LAPPACEUM L.) USING VITRIFICATION TECHNIQUE**

FLORENCE C. GINIBUN

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By

FLORENCE C. GINIBUN

**Thesis Submitted in Fulfilment of the Requirement for the
Degree of Master of Agricultural Science in the Faculty of Agriculture
Universiti Putra Malaysia**

February 2001



Dedicated To:

My Beloved Parents:

Camillus Ginibun and Jovina Polycarpus

My Beloved Sisters:

Janet, Rose, Rovina and Linda

My Beloved Relatives and Friends

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Agricultural Science.

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Chairman : Associate Professor Hor Yue Luan, Ph. D.

Faculty : Agriculture

The present study evaluates the effects of various loading solutions, concentrations of glycerol and vitrification solutions and their time of exposure on the vitrification of rambutan embryos in liquid nitrogen.

In the initial study to evaluate the effects of loading solutions on survival, excised embryos were exposed to four loading solutions. The two most promising loading solutions were LB (1.5 M glycerol + 0.4 M sucrose + 5 % DMSO), which gave 44.0 % viability and 32.4 % survival and LA (2.0 M glycerol + 0.4 M sucrose) which gave 39.3 % viability and 28.1 % survival after freezing.

The effects of different concentrations of glycerol (0 – 2.0 M) in the most promising loading solutions were evaluated further. For loading solution LB, 1.5 M glycerol gave highest survival of 22.7 %. For loading

solution LB, 1.5 M glycerol gave highest survival of 22.7 %. For loading solution LA, 0 M glycerol or the use of only 0.4 M sucrose gave the highest viability of 76.0 % and survival of 59.0 %. Hence, loading solution with only 0.4 M sucrose (LA without glycerol) was established in this study as the most effective loading solution for rambutan embryos.

The effects of exposure time (0 – 16 hours) to the best loading solution on survival of rambutan embryos were further investigated. It was found that 8 hours duration gave the highest viability (47.7 %) and survival (32.8 %).

Having confirmed the best loading treatment, the study further evaluates the effects of six vitrification solutions on survival of rambutan embryos in liquid nitrogen. The results show that after freezing, L Solution gave the highest viability (46.0 %) and survival (24.0 %). L Solution was therefore selected as the most effective vitrification solution.

In optimizing the time of exposure, excised rambutan embryos were exposed to L Solution for 0 to 90 minutes before LN exposure. The highest viability (55.6 %) and survival (40.3 %) after vitirification were achieved at 60 minutes exposure. Longer exposure to L Solution for up to 90 minutes reduced survival to 16.0 %.

This study concludes that 0.4 M sucrose loaded for 8 hours, followed by exposure to L Solution for 60 minutes was optimum for the vitrification of excised rambutan embryos, which yielded 55.6 % viability and 40.3 % survival.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains Pertanian

**PENKRIOWETAN EMBRIO RAMBUTAN
(*NEPHELIUM LAPPACEUM* L.) MELALUI TEKNIK VITRIFIKASI**

Oleh

FLORENCE C. GINIBUN

Februari 2001

Pengerusi Penyelia : Prof. Madya Hor Yue Luan, Ph.D.

Fakulti : Pertanian

Kajian ini menilai kesan pelbagai larutan 'loading', kepekatan larutan gliserol, larutan vitrifikasi dan tempoh pendedahannya ke atas penvitrifikasian embrio rambutan di dalam cecair nitrogen.

Dalam kajian menilai kesan larutan 'loading' ke atas kemandirian embrio rambutan, embrio didedahkan kepada empat larutan 'loading'. Dua jenis larutan 'loading' yang memberi kesan ialah LB (1.5 M gliserol + 0.4 M sukrosa + 5 % DMSO) di mana 44.0 % viabiliti dan 32.4 % kemandirian diperolehi dan LA (2.0 M gliserol + 0.4 M sukrosa) memberi hasil 39.3 % viability dan 28.1 % kemandirian setelah disejukk bekukan.

Kesan ke atas perbezaan kepekatan gliserol (0 – 2.0 M) dalam larutan 'loading' yang paling berpotensi dikaji seterusnya. Dalam larutan 'loading' LB, 1.5 M gliserol memberikan kemandirian yang tertinggi sebanyak 22.7 %. Dalam larutan 'loading' LA, 0 M gliserol atau

penggunaan hanya 0.4 M sukrosa, memberi viabiliti yang tertinggi sebanyak 76.0 % dan kemandirian sebanyak 59.0 %. Oleh yang demikian, larutan 'loading' dengan hanya 0.4 M sukrosa (LA tanpa gliserol) terbukti di dalam kajian ini sebagai larutan yang paling efektif terhadap embrio rambutan.

Kesan tempoh pendedahan (0 – 16 jam) larutan 'loading' yang terbaik ke atas kemandirian embrio rambutan seterusnya dinilai. Di dapati bahawa tempoh pendedahan selama 8 jam memberikan viabiliti (47.7 %) dan kemandirian (32.8 %) yang tertinggi.

Setelah mengenalpasti rawatan 'loading' yang terbaik, kajian seterusnya menilai kesan ke atas enam jenis larutan vitrifikasi terhadap kemandirian embrio rambutan dalam cecair nitrogen. Keputusan menunjukkan bahawa selepas penyejukbekuan, larutan L memberikan viabiliti (46.0 %) dan kemandirian (24.0 %) yang tertinggi dimana ianya lebih baik daripada larutan PVS2. Oleh yang demikian, larutan L dipilih sebagai larutan vitrifikasi yang paling efektif.

Untuk menentukan tempoh pendedahan yang optima, embrio rambutan dirawat dengan larutan L selama 0 sehingga 90 minit sebelum didedahkan ke dalam cecair nitrogen. Viabiliti (55.6 %) dan kemandirian (40.3 %) yang tertinggi selepas penvitrifikasikan diperolehi pada tempoh pendedahan 60 minit. Tempoh pendedahan yang lebih

panjang ke atas larutan L sehingga 90 minit mengurangkan kemandirian sebanyak 16.0 %.

Kajian dapat disimpulkan bahawa rawatan dengan 0.4 M sukrosa selama 8 jam diikuti dengan pendedahan kepada larutan L selama 60 minit adalah yang optima untuk penvitrifikasi embrio rambutan di mana menghasilkan sebanyak 55.6 % viability dan 40.3 % kemandirian.

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I certify that an Examination Committee met on 22nd February 2001 to conduct the final examination of Florence C. Ginibun on her Master of Agricultural Science thesis entitled "Cryopreservation of Excised Embryos of Rambutan (*Nephelium lappaceum* L.) Using Vitrification Technique" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Rajan Amartalingam, Ph.D.
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Hor Yue Luan, Ph.D.
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Saleh b. Kadzimin, Ph.D.
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

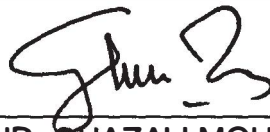
Baskaran Krishnapillay, Ph.D.
Forestry Field Division
Forestry Research Institute Malaysia (FRIM)
(Member)



MOHD. GHAZALI MOHAYIDIN, Ph.D.
Professor/Deputy Dean of Graduate School,
Universiti Putra Malaysia.

Date: 09 APR 2001

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of requirement for the degree of Master of Agricultural Science.



MOHD. GHAZALI MOHAYIDIN, Ph.D.
Professor/Deputy Dean of Graduate School,
Universiti Putra Malaysia.

Date:

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



FLORENCE C. GINIBUN

Date: 09.04.2001

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